Normative Values for Corneal Nerve Morphology Assessed Using Corneal Confocal Microscopy: A Multinational Normative Data Set

Diabetes Care 2015;38:838–843 | DOI: 10.2337/dc14-2311

Mitra Tavakoli,[1] Maryam Ferdousi,[1] Ioannis N. Petropoulos,[1] Julie Morris,[2] Nicola Pritchard,[3] Andrey Zhivov,[4] Dan Ziegler,[5,6] Danièle Pacaud,[7] Kenneth Romanchuk,[7] Bruce A. Perkins,[8] Leif E. Lovblom,[8] Vera Bril,[9] J. Robinson Singleton,[10] Gordon Smith,[10] Andrew J.M. Boulton,[1] Nathan Efron,[3] and Rayaz A. Malik[1,11]

OBJECTIVE

Corneal confocal microscopy is a novel diagnostic technique for the detection of nerve damage and repair in a range of peripheral neuropathies, in particular diabetic neuropathy. Normative reference values are required to enable clinical translation and wider use of this technique. We have therefore undertaken a multicenter collaboration to provide worldwide age-adjusted normative values of corneal nerve fiber parameters.

RESEARCH DESIGN AND METHODS

A total of 1,965 corneal nerve images from 343 healthy volunteers were pooled from six clinical academic centers. All subjects underwent examination with the Heidelberg Retina Tomograph corneal confocal microscope. Images of the central corneal subbasal nerve plexus were acquired by each center using a standard protocol and analyzed by three trained examiners using manual tracing and semiautomated software (CCMetrics). Age trends were established using simple linear regression, and normative corneal nerve fiber density (CNFD), corneal nerve fiber branch density (CNBD), corneal nerve fiber length (CNFL), and corneal nerve fiber tortuosity (CNFT) reference values were calculated using quantile regression analysis.

RESULTS

There was a significant linear age-dependent decrease in CNFD (−0.164 no./mm^2 per year for men, $P < 0.01$, and −0.161 no./mm^2 per year for women, $P < 0.01$). There was no change with age in CNBD (0.192 no./mm^2 per year for men, $P = 0.26$, and −0.050 no./mm^2 per year for women, $P = 0.78$). CNFL decreased in men (−0.045 mm/mm^2 per year, $P = 0.07$) and women (−0.060 mm/mm^2 per year, $P = 0.02$). CNFT increased with age in men (0.044 per year, $P < 0.01$) and women (0.046 per year, $P < 0.01$). Height, weight, and BMI did not influence the 5th percentile normative values for any corneal nerve parameter.

CONCLUSIONS

This study provides robust worldwide normative reference values for corneal nerve parameters to be used in research and clinical practice in the study of diabetic and other peripheral neuropathies.

[1]Centre for Endocrinology and Diabetes, Institute of Human Development, University of Manchester, Manchester, U.K.
[2]Medical Statistics Unit, University of Manchester and University Hospital of South Manchester, Manchester, U.K.
[3]Institute of Health Biomedical Innovation, Queensland University of Technology, Brisbane, Australia
[4]Department of Ophthalmology, University of Rostock, Rostock, Germany
[5]Institute for Clinical Diabetology, German Diabetes Center at Heinrich Heine University, Leibniz Center for Diabetes Research, Düsseldorf, Germany
[6]Department of Endocrinology and Diabetology, University Hospital, Düsseldorf, Germany
[7]Alberta Children's Hospital, Alberta Children's Hospital Research Institute, University of Calgary, Calgary, Canada
[8]Division of Endocrinology, Leadership Sinai Centre for Diabetes, University of Toronto, Toronto, Canada
[9]Division of Neurology, University Health Network, University of Toronto, Toronto, Canada
[10]Department of Neurology, University of Utah, Salt Lake City, UT
[11]Weill Cornell Medical College in Qatar, Ar Rayyān, Qatar

Corresponding author: Rayaz A. Malik, rayaz.a.malik@man.ac.uk.

Received 1 October 2014 and accepted 4 January 2015.

This article contains Supplementary Data online at http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc14-2311/-/DC1.

The use of corneal confocal microscopy (CCM) for rapid, noninvasive clinical assessment of corneal nerves has grown substantially in recent years (1). It has proven to be particularly useful as a diagnostic marker for the detection of diabetic neuropathy (2–6) and a range of other peripheral neuropathies (7–10).

However, the clinical translation of CCM has been limited by a lack of normative reference values, allowing investigators to define pathological changes. Furthermore, there is considerable inconsistency in the literature with regard to the density of subbasal nerves in the cornea in control subjects based on the type of instrument, protocol used to acquire images, and definition of corneal nerve structures (3,11). Studies using laser scanning confocal microscopy have reported higher densities of subbasal corneal nerves compared with studies using the tandem scanning confocal microscope and slit scanning confocal microscope, due to differences in the light source, contrast, and resolution of these instruments (11). Furthermore, a range of methods has been used to capture and quantify the nerves and differed in terms of the site and number of images selected and analyzed per subject (12). The majority of studies have defined subbasal nerve density as the total number of major nerves in an area of corneal tissue (no./mm^2) (3,13). However, others have presented the data as the number of nerves per image (14) or the total length of the nerves within a frame (15,16) and still referred to it as a density.

Aging and sex may impact peripheral nerve morphology. Corneal sensitivity has been shown to decline with age (17). However, while some relatively small studies have demonstrated no correlation between corneal nerve density and age (4,15,16,18), other earlier studies demonstrated a significant reduction with age (16,19). Using a definition of nerve density that is consistent with our definition of corneal nerve fiber length (CNFL), a study of 85 healthy control subjects showed a linear decline in subbasal nerve density of 0.9% per year (20).

The aim of the present international multicenter collaboration was to establish worldwide age-adjusted normative values of corneal nerve fiber parameters using a common adopted method to capture the images: the same CCM instrument and manual analysis using strict definitions of corneal nerve fiber parameters (3,21).

RESEARCH DESIGN AND METHODS

Six independent international study groups who have previously reported normal values for corneal nerve parameters using the Heidelberg Retina Tomograph (HRT III) Rostock Cornea module (RCM) were invited to provide the coordinating center (Manchester, U.K.) with CCM images of corneal nerves from their healthy subjects who were enrolled and had consented and been studied previously. Corneal nerve images from 343 healthy volunteers were pooled from each academic center (Manchester, *n* = 139; Brisbane, *n* = 59; Utah, *n* = 48; Düsseldorf, *n* = 42; Calgary, *n* = 41; and Toronto, *n* = 14).

Eligibility criteria for inclusion of subjects as control subjects in the original study from each center were good health, age between 5 and 85 years, and lack of neurological signs or symptoms or conditions known to cause neuropathy including diabetes, impaired glucose tolerance, metabolic syndrome, vitamin B_{12} deficiency, and idiopathic small fiber neuropathy. Subjects with a history of using hard contact lenses and previous refractive surgery were excluded. Data on height, weight, BMI, and HbA_{1c} were recorded where available. In each center, informed written consent was obtained from all participants prior to their enrolment in the study. All control subjects were recruited and assessed; as part of ongoing clinical studies at each center, they underwent detailed evaluation of neurological symptoms and deficits, quantitative sensory testing, and electrophysiology as well as standard blood tests to exclude other causes of neuropathy, which included serum B_{12}, folate, antinuclear antibody (ANA), and immunoglobulins. This research adhered to the tenets of the Declaration of Helsinki, and local ethics committee approval was obtained in all centers.

Subjects underwent examination with the HRT III Rostock Cornea module in vivo corneal confocal microscope (IVCCM). The subject's eyes were anesthetized using a drop of 0.4% Benoxinate hydrochloride, and Viscotears was applied on the front of the eye for lubrication. A drop of viscoelastic gel was placed on the tip of the objective lens, and a sterile disposable Perspex cap was placed over the lens allowing optical coupling of the objective lens to the cornea. The patient was instructed to fixate on a target with the eye not being examined. Several scans of the entire depth of the cornea were recorded by turning the fine focus of the objective lens backward and forward for ~2 min using the section mode, which enables manual acquisition and storage of single images of all corneal layers. This provides en face two-dimensional images with a lateral resolution of ~2 μm/pixel and a final image size of 400 × 400 pixels of the subbasal nerve plexus of the cornea from each control subject. This layer is of particular relevance for defining neuropathic changes, since it is the location of the main nerve plexus that supplies the overlying corneal epithelium. Each nerve fiber bundle contains unmyelinated fibers, which run parallel to Bowman layer before dividing and terminating as individual axons in the surface epithelium. Three to six images from the center of the cornea of both eyes were selected and examined in a masked fashion. Three experienced examiners (M.T., M.F., and I.N.P.), masked from the clinical details of the subjects, quantified 1,965 images of all study participants using purpose-written, proprietary software (CCMetrics [M.A. Dabbah, Imaging Science, University of Manchester]). Four corneal nerve parameters were quantified: *1*) corneal nerve fiber density (CNFD) (the total number of major nerves per millimeter squared of corneal tissue), *2*) corneal nerve branch density (CNBD) (the number of branches emanating from all major nerve trunks per millimeter squared of corneal tissue), *3*) CNFL (the total length of all nerve fibers and branches within the area of corneal tissue), and *4*) corneal nerve fiber tortuosity (CNFT) (tortuosity coefficient [TC]), which represents the degree of tortuosity from a straight line joining the ends of each main nerve fiber.

Statistical Analysis

Data are presented as mean (SD), median, or quantile for men and women by each age decade. Age trends in men and women were investigated separately using simple linear regression analyses. Quantile regression was carried out to determine normative reference values for

Table 1—Demographic data

	Age-groups (years)						
	<16	16–25	26–35	36–45	46–55	56–65	>66
Number (female/male)	19/18	28/32	26/23	26/17	34/25	22/32	16/25
Age (years)	12.5 (1.8)	20.8 (3.1)	29.9 (2.9)	41.4 (3.0)	49.8 (2.7)	60.7 (2.9)	70.3 (3.6)
Height (m)	1.54 (0.12)	1.69 (0.09)	1.69 (0.08)	1.68 (0.09)	1.68 (0.10)	1.72 (0.10)	1.70 (0.09)
Weight (kg)	46.8 (11.9)	64.9 (12.6)	68.7 (12.7)	73.5 (12.6)	79.8 (18.5)	78.1 (14.9)	76.4 (11.9)
BMI (kg/m^2)	19.5 (2.5)	22.4 (3.7)	24.2 (3.2)	25.0 (4.3)	28.0 (5.8)	26.1 (4.0)	25.9 (2.8)
HbA_{1c} (%)	—	5.26 (0.30)	4.82 (1.50)	5.08 (1.61)	5.06 (1.62)	4.96 (1.74)	5.41 (1.38)
HbA_{1c} (mmol/mol)	—	34.02 (3.2)	34.19 (3.5)	36.75 (3.6)	37.25 (4.3)	37.23 (2.6)	38.73 (4.1)

Data are mean (SD) unless otherwise indicated.

CNFD, CNBD, CNFL, and CNFT. This technique allows quantiles to be estimated as a smooth function of age without imposing a parametric distribution, and this method is particularly robust in the presence of outliers.

RESULTS

Of the subjects from six centers that participated in this study, providing IVCCM images of the corneal subbasal nerves from 343 healthy subjects aged between 9 and 82 years old, 172 were male and 171 were female. The demographic results of the participants are presented in Table 1. HbA_{1c} was available for 133 subjects, and BMI, height, and weight were available for 266 subjects (Table 1). HbA_{1c}, height, weight, and BMI did not influence the 5th percentile normative values for CNFD, CNBD, CNFL, or CNFT.

Representative images for each age-group are presented in Fig. 1. Median values for corneal nerve parameters are presented in Table 2 for female and male subjects. Fifth quantile values for CNFD, CNBD, and CNFL and 95th quantiles for CNFT are presented in Table 3. There was a significant linear age-dependent decrease in CNFD (-0.164 no./mm^2 per year for men, $P < 0.01$, and -0.161 no./mm^2 per year for women, $P < 0.01$). There was no change in CNBD (0.192 no./mm^2 per year for men, $P = 0.26$, and -0.050 no./mm^2 per year for women, $P = 0.78$). CNFL decreased in men (-0.045 mm/mm^2 per year, $P = 0.07$) and women (-0.060 mm/mm^2 per year, $P = 0.02$). CNFT increased in men (0.044 per year, $P < 0.01$) and women (0.046 per year, $P < 0.01$).

Regression plots for each corneal nerve fiber parameter with lines denoting the median and the 5th and the 95th percentiles are shown in Fig. 2. The findings were similar regardless of sex for CNFD ($R^2 = 0.11$, slope loss % per year -0.161, $P < 0.0001$), CNBD ($R^2 = 0.005$, slope loss % per year -0.074, $P = 0.55$), CNFL ($R^2 = 0.026$, slope loss % per year -0.052, $P = 0.003$), and CNFT ($R^2 = 0.052$, slope increase % per year 0.044, $P < 0.0001$).

CONCLUSIONS

IVCCM has evolved rapidly from a technique predominantly used in clinical research to a diagnostic tool for a variety of ophthalmic and neurological applications (1). It has now emerged as a powerful technique for the study of corneal cellular structure in health and disease in patients (22) and in animal models of diabetic neuropathy (23). The noninvasive nature of this technique has made it an ideal tool to examine all microstructures of the cornea (24).

There is a burgeoning literature on the use of CCM to quantify peripheral neuropathies, in particular diabetic neuropathy (2–6). Thus, CCM has been shown to be an accurate noninvasive method for the early diagnosis of diabetic neuropathy (3,25), idiopathic small-fiber neuropathy (26), Fabry disease (9), hereditary sensory and autonomic neuropathy (8), autoimmune neuropathy (27), and neuropathy associated with Crohn disease and chemotherapy (28). It has been shown to have high reproducibility (29,30) and repeatability (32), with good sensitivity and specificity for the diagnosis of diabetic neuropathy (3,32).

However, before CCM can be adopted for wider clinical use, there is clearly a need to standardize the method of capturing, sampling, and analyzing the images. And there is a need to adopt a global standard approach to quantify corneal nerve morphology using rigorous definitions for each morphologic parameter to enable comparison between different studies. The use of standardized manual analysis software would resolve this problem partially, but of course interobserver differences would remain, based on difference between observers in the recognition of corneal nerve structures (31). This can be overcome with the use of automated image

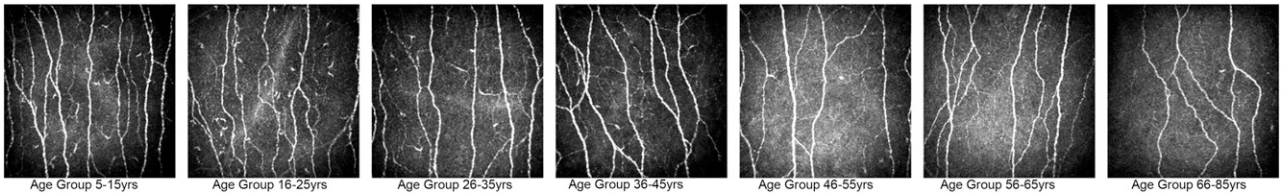

Figure 1—Representative CCM images from the subbasal plexus per age-group. yrs, years.

Table 2—Corneal nerve normative values

	Female subjects					Male subjects				
Age (years)	No. of subjects	Median CNFD (no./mm^2)	Median CNBD (no./mm^2)	Median CNFL (mm/mm^2)	Median CNFT (TC)	No. of subjects	Median CNFD (no./mm^2)	Median CNBD (no./mm^2)	Median CNFL (mm/mm^2)	Median CNFT (TC)
<16	19	33.33	87.60	26.43	13.17	18	34.13	91.62	24.56	13.49
16–25	28	31.85	77.01	25.45	13.77	32	32.44	53.68	23.16	13.92
26–35	26	30.20	68.46	24.37	14.43	23	30.56	58.14	22.92	14.40
36–45	26	28.56	63.27	23.28	15.09	17	28.68	71.93	23.34	14.87
46–55	34	26.91	61.46	22.20	15.75	25	26.80	81.05	23.63	15.35
56–65	22	25.27	63.02	21.11	16.41	32	24.92	79.17	23.03	15.83
>65	16	23.54	68.28	19.97	17.10	25	22.95	61.68	20.61	16.33

Data are median values per age span.

analysis software that has been developed recently to rapidly quantify corneal nerve pathology (33,34).

As stated earlier, while some relatively small studies have demonstrated no correlation between corneal nerve density and age (4,15,16,18), other earlier studies demonstrated a significant reduction with age (16,19). The disagreement between these previous studies can be potentially explained by the types of corneal confocal microscopes (tandem scanning confocal microscope, slit scanning confocal microscope, and laser scanning corneal confocal microscope) that have been used to acquire images and also the method (volume scan, section scan) used to capture the images as well as the parameters measured. The current data set shows that corneal nerve morphology behaves very similarly to intraepidermal nerve fiber density (IENFD) in terms of an age-dependent decrease in nerve fiber density (35). Furthermore, we show an age-dependent reduction in CNFL and an increase in CNFT but no significant effect on CNBD, presumably from increases as well as decreases in nerve branches due to concomitant degeneration and regeneration, respectively. The possible effects of weight and indeed BMI have been suggested as possible confounders when undertaking longitudinal studies using IENFD (36). Thus, it is reassuring that like IENFD (35), corneal nerve fiber parameters also showed no effect of height, weight, or BMI. A previous study in 64 healthy volunteers showed that CNFL had a broad distribution and was not related to age, although their age range was limited to 39 $\pm$ 18 years. Furthermore, multivariate regression analysis demonstrated that HbA_{1c} was the only independent variable accounting for the variation in CNFL (37). Indeed, we also show a broad distribution for corneal nerve parameters, but this is primarily explained by an effect of age. This age effect may be relevant when interpreting recent data showing a more marked reduction in CNFL in patients with type 1 diabetes and paradoxically minimal change in patients with type 2 diabetes and mild neuropathy as opposed to those without and with severe neuropathy (6). Significant changes have been found in patients recently diagnosed with type 2 diabetes (5) and subjects with impaired glucose tolerance (38).

We acknowledge some limitations in the current study including an unequal number of subjects in each group. We also do not have complete data sets for HbA_{1c} or oral glucose tolerance test or data on smoking, alcohol intake, or actual levels of vitamin B_{12} or folate. However, the latter were checked in each center, and any individuals with an abnormality were excluded.

In conclusion, the current study represents the largest multicenter cohort of healthy control subjects who have undergone CCM using a standardized technique to capture central corneal images with the same model of microscope and rigorous quantification of corneal nerve morphology in one center using a standard definition. We show an age-dependent decrease in CNFD and CNFL with an increase in CNFT. We additionally provide age-dependent normative cutoffs to aid clinicians to identify pathological reductions,

Table 3—Corneal nerve parameter cutoff points for clinical use

	Female subjects				Male subjects			
Age (years)	0.05th quantile CNFD (no./mm^2)	0.05th quantile CNBD (no./mm^2)	0.05th quantile CNFL (mm/mm^2)	0.95th quantile CNFT (TC)	0.05th quantile CNFD (no./mm^2)	0.05th quantile CNBD (no./mm^2)	0.05th quantile CNFL (mm/mm^2)	0.95th quantile CNFT (TC)
<16	23.98	46.52	19.19	17.23	22.18	36.80	18.82	17.09
16–25	20.07	34.01	15.08	21.06	17.96	20.62	15.93	19.66
26–35	16.85	24.04	13.17	22.76	14.54	16.49	14.05	21.04
36–45	14.79	18.19	12.48	22.79	12.46	15.78	13.20	21.86
46–55	13.91	16.47	12.48	22.16	11.71	15.55	13.01	22.43
56–65	14.20	18.89	12.90	21.91	12.29	14.85	13.12	22.85
>65	15.77	25.86	13.67	23.17	14.35	13.23	13.15	23.20

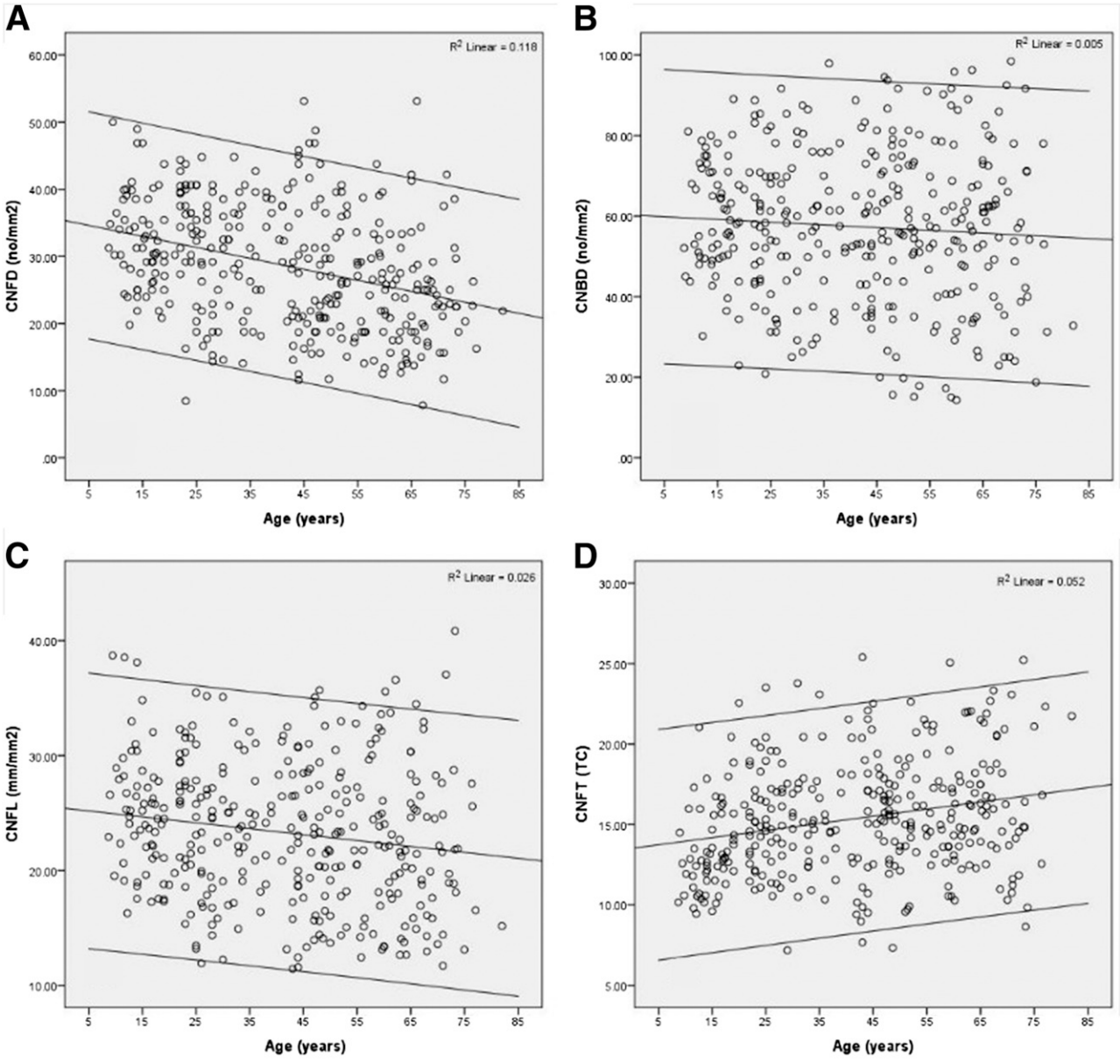

Figure 2—Scatterplot showing CNFD (*A*), CNBD (*B*), CNFL (*C*), and CNFT (*D*) in 343 healthy individuals. Lines depict 5th, 50th, and 95th percentiles (unadjusted).

sufficient to identify significant nerve damage and, hence, diagnose peripheral neuropathy. These data provide the basis for wider use of CCM as a diagnostic test for diabetic and other peripheral neuropathies.

Funding. The centers were supported by grants as follows. Manchester: This research was funded by the National Institutes of Health (R1-05991), JDRF (R106784), and JDRF (27-2008- 362). Support from the National Institute for Health Research–Wellcome Trust Clinical Research Facility and the Collaboration for Leadership in Applied Health Research and Care Greater Manchester is acknowledged. Brisbane: This research was funded by JDRF (8-2008-362). Utah: This research was funded by R01-DK064814 and by the National Center for Advancing Translational Sciences of the National Institutes of Health (1ULTR001067). Düsseldorf: This work was supported by the Ministry of Science and Research of the State of North Rhine–Westphalia and the German Federal Ministry of Health. Calgary: Part of this work was supported by JDRF (17-2008-1032). Toronto: This research was funded by JDRF (17-2008-715) and Canadian Diabetes Association operating grant OG-3-3123-BP.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. M.T. designed the study, researched data for Manchester, analyzed CCM images and data for all centers, and wrote the manuscript. M.F. and I.N.P. researched data for Manchester and analyzed CCM images for all centers. J.M. was the statistical consultant for the study and analyzed data for all centers. N.P. researched data for Brisbane. A.Z. and D.Z. researched data for Düsseldorf. D.P. and K.R. researched data for Calgary. B.A.P., L.E.L., and V.B. researched data for Toronto. J.R.S. and G.S. researched data for Utah. A.J.M.B. reviewed and revised the manuscript. N.E. reviewed and revised the manuscript. R.A.M. designed the study, supervised the project, and reviewed and revised the manuscript. R.A.M. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Tavakoli M, Petropoulos IN, Malik RA. Assessing corneal nerve structure and function in diabetic neuropathy. Clin Exp Optom 2012;95:338–347
2. Edwards K, Pritchard N, Vagenas D, Russell A, Malik RA, Efron N. Utility of corneal confocal microscopy for assessing mild diabetic neuropathy: baseline findings of the LANDMark study. Clin Exp Optom 2012;95:348–354
3. Tavakoli M, Quattrini C, Abbott C, et al. Corneal confocal microscopy: a novel noninvasive test to diagnose and stratify the severity of human diabetic neuropathy. Diabetes Care 2010;33:1792–1797
4. Wu T, Ahmed A, Bril V, et al. Variables associated with corneal confocal microscopy parameters in healthy volunteers: implications for diabetic neuropathy screening. Diabet Med 2012;29:e297–e303
5. Ziegler D, Papanas N, Zhivov A, et al.; German Diabetes Study (GDS) Group. Early detection of nerve fiber loss by corneal confocal microscopy and skin biopsy in recently diagnosed type 2 diabetes. Diabetes 2014;63:2454–2463
6. Stem MS, Hussain M, Lentz SI, et al. Differential reduction in corneal nerve fiber length in patients with type 1 or type 2 diabetes mellitus. J Diabetes Complications 2014;28:658–661
7. Tavakoli M, Kallinikos P, Iqbal A, et al. Corneal confocal microscopy detects improvement in corneal nerve morphology with an improvement in risk factors for diabetic neuropathy. Diabet Med 2011;28:1261–1267
8. Tavakoli M, Marshall A, Banka S, et al. Corneal confocal microscopy detects small-fiber neuropathy in Charcot-Marie-Tooth disease type 1A patients. Muscle Nerve 2012;46:698–704
9. Tavakoli M, Marshall A, Thompson L, et al. Corneal confocal microscopy: a novel noninvasive means to diagnose neuropathy in patients with Fabry disease. Muscle Nerve 2009;40:976–984
10. Campagnolo M, Lazzarini D, Fregona I, et al. Corneal confocal microscopy in patients with oxaliplatin-induced peripheral neuropathy. J Peripher Nerv Syst 2013;18:269–271
11. Tavakoli M, Malik RA. Corneal confocal microscopy: a novel non-invasive technique to quantify small fibre pathology in peripheral neuropathies. J Vis Exp 2011;47:2194
12. Vagenas D, Pritchard N, Edwards K, et al. Optimal image sample size for corneal nerve morphometry. Optom Vis Sci 2012;89:812–817
13. Messmer EM, Schmid-Tannwald C, Zapp D, Kampik A. In vivo confocal microscopy of corneal small fiber damage in diabetes mellitus. Graefes Arch Clin Exp Ophthalmol 2010;248:1307–1312
14. Midena E, Brugin E, Ghirlando A, Sommavilla M, Avogaro A. Corneal diabetic neuropathy: a confocal microscopy study. J Refract Surg 2006;22(Suppl.):S1047–S1052
15. Erie JC, McLaren JW, Hodge DO, Bourne WM. The effect of age on the corneal subbasal nerve plexus. Cornea 2005;24:705–709
16. Grupcheva CN, Wong T, Riley AF, McGhee CN. Assessing the sub-basal nerve plexus of the living healthy human cornea by in vivo confocal microscopy. Clin Experiment Ophthalmol 2002;30:187–190
17. Roszkowska AM, Colosi P, Ferreri FM, Galasso S. Age-related modifications of corneal sensitivity. Ophthalmologica 2004;218:350–355
18. Patel DV, Tavakoli M, Craig JP, Efron N, McGhee CN. Corneal sensitivity and slit scanning in vivo confocal microscopy of the subbasal nerve plexus of the normal central and peripheral human cornea. Cornea 2009;28:735–740
19. Oliveira-Soto L, Efron N. Morphology of corneal nerves using confocal microscopy. Cornea 2001;20:374–384
20. Niederer RL, Perumal D, Sherwin T, McGhee CN. Age-related differences in the normal human cornea: a laser scanning in vivo confocal microscopy study. Br J Ophthalmol 2007;91:1165–1169
21. Tavakoli M, Mitu-Pretorian M, Petropoulos IN, et al. Corneal confocal microscopy detects early nerve regeneration in diabetic neuropathy after simultaneous pancreas and kidney transplantation. Diabetes 2013;62:254–260
22. Patel DV, McGhee CN. In vivo confocal microscopy of human corneal nerves in health, in ocular and systemic disease, and following corneal surgery: a review. Br J Ophthalmol 2009;93:853–860
23. Malik RA. From the bedside to the bench and back again, with corneal confocal microscopy. Invest Ophthalmol Vis Sci 2014;55:1231
24. Bitirgen G, Ozkagnici A, Malik RA, Kerimoglu H. Corneal nerve fibre damage precedes diabetic retinopathy in patients with type 2 diabetes mellitus. Diabet Med 2014;31:431–438
25. Rosenberg ME, Tervo TM, Immonen IJ, Müller LJ, Grönhagen-Riska C, Vesaluoma MH. Corneal structure and sensitivity in type 1 diabetes mellitus. Invest Ophthalmol Vis Sci 2000;41:2915–2921
26. Tavakoli M, Marshall A, Pitceathly R, et al. Corneal confocal microscopy: A novel means to detect nerve fibre damage in idiopathic small fibre neuropathy. Exp Neurol 2010;223:245–250
27. Lalive PH, Truffert A, Magistris MR, Landis T, Dosso A. Peripheral autoimmune neuropathy assessed using corneal in vivo confocal microscopy. Arch Neurol 2009;66:403–405
28. Ferrari G, Gemignani F, Macaluso C. Chemotherapy-associated peripheral sensory neuropathy assessed using in vivo corneal confocal microscopy. Arch Neurol 2010;67:364–365
29. Efron N, Edwards K, Roper N, et al. Repeatability of measuring corneal subbasal nerve fiber length in individuals with type 2 diabetes. Eye Contact Lens 2010;36:245–248
30. Hertz P, Bril V, Orszag A, Ahmed A, Ng E, Nwe P, et al. Reproducibility of in vivo corneal confocal microscopy as a novel screening test for early diabetic sensorimotor polyneuropathy. Diabet Med 2011;28:1253–1260
31. Petropoulos IN, Manzoor T, Morgan P, et al. Repeatability of in vivo corneal confocal microscopy to quantify corneal nerve morphology. Cornea 2013;32:e83–e89
32. Ahmed A, Bril V, Orszag A, et al. Detection of diabetic sensorimotor polyneuropathy by corneal confocal microscopy in type 1 diabetes: a concurrent validity study. Diabetes Care 2012;35:821–828
33. Scarpa F, Grisan E, Ruggeri A. Automatic recognition of corneal nerve structures in images from confocal microscopy. Invest Ophthalmol Vis Sci 2008;49:4801–4807
34. Dabbah MA, Graham J, Petropoulos IN, Tavakoli M, Malik RA. Automatic analysis of diabetic peripheral neuropathy using multi-scale quantitative morphology of nerve fibres in corneal confocal microscopy imaging. Med Image Anal 2011;15:738–747
35. Lauria G, Bakkers M, Schmitz C, et al. Intraepidermal nerve fiber density at the distal leg: a worldwide normative reference study. J Peripher Nerv Syst 2010;15:202–207
36. Dyck PJ, Dyck PJ, Klein CJ, Weigand SD. Does impaired glucose metabolism cause polyneuropathy? Review of previous studies and design of a prospective controlled population-based study. Muscle Nerve 2007;36:536–541
37. Wu T, Ahmed A, Bril V, et al. Variables associated with corneal confocal microscopy parameters in healthy volunteers: implications for diabetic neuropathy screening. Diabet Med 2012;29:e297–e303
38. Asghar O, Petropoulos IN, Alam U, et al. Corneal confocal microscopy detects neuropathy in subjects with impaired glucose tolerance. Diabetes Care 2014;37:2643–2646